Published in final edited form as:

Neurobiol Dis. 2007 July ; 27(1): 1–10. doi:10.1016/j.nbd.2007.04.006.

Functional *MAPT* haplotypes: Bridging the gap between genotype and neuropathology

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Abstract

The microtubule associated protein tau (*MAPT*) locus has long been associated with sporadic neurodegenerative disease, notably progressive supranuclear palsy and corticobasal degeneration, and more recently with Alzheimer's disease and Parkinson's disease. However, the functional biological mechanisms behind the genetic association have only now started to emerge. The genomic architecture in the region spanning *MAPT* is highly complex, and includes a ~1.8 Mb block of linkage disequilibrium (LD). The region is divided into two major haplotypes, H1 and H2, defined by numerous single nucleotide polymorphisms and a 900 kb inversion which suppresses recombination. Fine mapping of the *MAPT* region has identified sub-clades of the *MAPT*H1 haplotype which are specifically associated with neurodegenerative disease. Here we briefly review the role of *MAPT* in sporadic and familial neurodegenerative disease, and then discuss recent work which, for the first time, proposes functional mechanisms to link *MAPT* haplotypes with the neuropathology seen in patients.

Keywords

MAPT; H1 haplotype; progressive supranuclear palsy; tauopathy; splicing; gene expression; functional polymorphisms; susceptibility mechanisms

Introduction

Future demographic projections are predicting the number of people aged 60 years or greater to reach nearly 1.2 billion by 2025 (<http://www.who.int/ageing/en/>). Correspondingly, this will increase the prevalence of diseases affecting ageing populations such as the neurodegenerative dementias and movement disorders. It is estimated that 24 million people currently have dementia worldwide and that this number will double to 42 million by 2020 (Ferri et al. 2005). Dementias and movement disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) are expected to surpass cancer as the second most common cause of death by 2040 (World Health Organization, 2004). It is becoming clear that neurodegenerative diseases affecting ageing populations are an increasingly important public health concern.

Common to many neurodegenerative diseases is the accumulation of abnormal protein in cells affected by neurodegeneration. Characteristic neuropathological aggregations are seen in PD in which aggregates of α -synuclein form Lewy-bodies and in AD where A β peptides form neuritic plaques. Intracellular aggregations of abnormally hyperphosphorylated

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microtubule associated protein tau (tau), known as neurofibrillary tangles (NFTs), are the major pathological feature of tauopathies, a diverse group of neurodegenerative dementias and movement disorders. Tauopathies include diseases such as progressive supranuclear palsy (PSP), AD, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), corticobasal degeneration (CBD), argyrophilic grain disease (AGD) and Pick's disease (PiD). Identification of tau as the major component in neurofibrillary tangles positioned the *MAPT* locus as a leading causal candidate gene in these neurodegenerative diseases.

The *MAPT* gene locus is located on chromosome 17q21 and consists of 16 exons (Andreadis et al. 1992) (Fig. 1). Tau protein is expressed predominantly in the neurons of the peripheral and central nervous systems where it has a role in building and stabilizing microtubules, neuronal polarity and signal transduction (for review see (Shahani and Brandt 2002)). In the adult human central nervous system, six protein isoforms are generated by alternative splicing of exons 2, 3 and 10 (Goedert et al. 1988; Goedert et al. 1989; Goedert et al. 1989; Andreadis et al. 1992) (Fig. 1). Exons 2 and 3 code for short amino terminal inserts that form part of the acidic projection domain that may interact with the plasma membrane (Brandt et al. 1995) and regulate spacing between microtubules (Chen et al. 1992). The interaction of tau with microtubules is mediated by the microtubule binding domains formed by imperfect repeats of 31 or 32 amino acids at the carboxyl terminus, encoded by exons 9 – 12 (Goedert et al. 1988; Gustke et al. 1994; Trinczek et al. 1995). The alternative splicing of exon 10 generates proteins with either three (exon 10–; 3R tau) or four (exon 10+; 4R tau) microtubule binding domains (Fig. 1).

Neurofibrillary tangles are found in AD, PSP, FTDP-17, CBD, AGD and PiD. The filamentous tau that aggregates to form NFTs in AD consists mainly of paired helical filaments 10 – 20 nm in diameter (Kidd 1963; Crowther 1990) whereas the PSP tangles are composed of 15 – 18 nm diameter straight filaments (Tellez-Nagel and Wisniewski 1973; Powell et al. 1974). Tangle isoform composition differs between tauopathies, being comprised of both 3R tau and 4R tau in AD (Sergeant et al. 1997) and predominately 3R tau in PiD (Delacourte et al. 1996). The major component of the insoluble tangles in PSP, CBD, AGD and FTDP-17 is 4R tau (Buee Scherrer et al. 1996; Arai et al. 2001; Togo et al. 2002). Consequently, these diseases are referred to as 4R tauopathies.

In 1998 the discovery of multiple *MAPT* mutations in FTDP-17 provided the first evidence that changes in tau alone could cause neurodegenerative disease. In addition, the FTDP-17 splice site mutations within *MAPT* that increase the inclusion of exon 10 in transcripts show an imbalance in the ratio of 3R and 4R tau isoforms is sufficient to cause disease (Hutton et al. 1998; Spillantini et al. 1998; D'Souza et al. 1999). In depth analysis of the region around exon 10 containing these rare pathogenic mutations has uncovered several functional cis-elements in the *MAPT* sequence which may give insight in to how intronic and non-coding mutations or polymorphisms may cause sporadic tauopathies.

Cis-elements and trans-acting factors affect splicing

FTDP-17 mutations reveal *MAPT* regulatory splicing sequences

The alternative splicing of *MAPT* exon 10 is moderated in part by the nature of the intronic sequences upstream and downstream of the exon (Gao et al. 2000). Neither the 3' nor the 5' exon 10 splice sites conform to the splicing consensus sequence, differing from the canonical sites by one and four nucleotides, respectively, (Andreadis et al. 1992) resulting in weak binding of the U1 snRNP (Hutton et al. 1998; Spillantini et al. 1998; Jiang et al. 2000). In vivo, the 5' splice site is strengthened by mutations S305N and exon 10 +3, resulting in increased exon 10 inclusion in transcripts (Hutton et al. 1998; Spillantini et al. 1998) (Fig.

2A). Weak splice sites around exon 10 may allow subtle spatial and temporal regulation of 3R and 4R isoforms, by allowing splicing regulation through a collection of local splicing enhancer and silencer elements.

Systematic deletions across exon 10 have revealed that nearly all the exon is involved in splicing regulation (D'Souza and Schellenberg 2000) (Fig. 2A). At the 5' of exon 10, three exonic splicing enhancers have been identified: a SC35-like enhancer, a polypurine enhancer (PPE) and an AC-rich element (ACE) (D'Souza et al. 1999; D'Souza and Schellenberg 2000).

The polypurine enhancer has a high purine content and is the region affected by the mutations N279K and K280del. N279K enhances exon 10 splicing by adding an extra AAG-repeat that strengthens the PPE (D'Souza et al. 1999; Hasegawa et al. 1999). In contrast, the K280del abolishes exon 10 splicing by removing an AAG-repeat (D'Souza et al. 1999; Rizzu et al. 1999). Trans-acting factors that bind the PPE include SF2/ASF, Tra2 β , SRp30c and SRp54 (Jiang et al. 2003; Kondo et al. 2004; D'Souza and Schellenberg 2006).

Downstream of the PPE is a putative A/C rich enhancer. The deletion of nucleotides in this region results mainly in decreased inclusion of exon 10 in transcripts. This region is affected by the silent mutation CTT>CTC (L284L) that is thought to exert its effect by increasing the content of AC nucleotides (D'Souza et al. 1999; D'Souza and Schellenberg 2000).

Further downstream of these enhancers, three different pathogenic mutations have been found at the same codon. The missense N296H and silent AAT > AAC (N296N) mutations enhance exon 10 inclusion, while a deletion at the same codon (N296del) is either neutral or enhances exon 10 splicing (Spillanti et al. 2000; Grover et al. 2002; Yoshida et al. 2002). These mutations have been proposed to either disrupt a 18-nucleotide silencer or change the silencer into an enhancer (D'Souza and Schellenberg 2000).

Alternative splicing regulation at the 5' splice site – Stem-loop theory

Mutations in the *MAPT* exon 10 5' splice site have highlighted the silencing function of this region on exon 10 inclusion. One theory proposes that these mutations disrupt a stem-loop structure that blocks U1 snRNP or another factor from binding, thus preventing inclusion of exon 10 in transcripts (Hutton et al. 1998; Spillanti et al. 1998) (Fig. 2B). Mutations within the predicted stem increase inclusion of exon 10 in transcripts, while mutations generated in the putative loop have no effect on splicing (Grover et al. 1999). In addition, stabilizing the stem loop by either inserting extra bases to elongate the stem or by generating more stable pairing along the stem, leads to a decrease in exon 10 inclusion (Grover et al. 1999; Donahue et al. 2006). Stem-loop secondary structures have been indicated through in vitro gel migration assays (Grover et al. 1999), by exposure of transcripts to RNase H (Jiang et al. 2000) and by UV melting experiments (Donahue et al. 2006).

While secondary structures can be observed in in vitro experiments (Grover et al. 1999; Varani et al. 1999), it is not clear if the same structure would form in vivo because in vivo there are multiple splicing regulatory proteins that coat the pre-mRNA possibly preventing secondary structures from forming (D'Souza and Schellenberg 2000). Additionally, the stem-loop structures proposed are often ambiguous and do not agree between groups (Fig 2B). Importantly, regulation through the stem loop structure does not necessarily allow for regulation by trans-acting factors that may affect differential splicing seen throughout development.

Alternative splicing regulation at the 5' splice site – Linear sequence theory

An alternative hypothesis is that the inhibitory sequence in intron 10 is a linear cis-acting element that binds trans-acting splicing factors (D'Souza et al. 1999; D'Souza and Schellenberg 2000) (Fig. 2C). Some of the mutations that destabilize the stem-loop structure may also function to increase exon 10 inclusion by increasing affinity for the U1 and U6 snRNPs which have both been implicated in exon 10 splicing inclusion. A proposed intronic silencer at position exon 10 +11 to exon 10 +18 retains its function even after it has been translocated to different positions, including a position within exon 10 as well as a heterologous setting, where the complementary bases are not present to form a stem loop structure (D'Souza and Schellenberg 2002). An intronic splicing modulator located at position exon 10 +19 to exon 10 +26, acts in conjunction with the intronic splicing silencer to regulate the splicing of exon 10 (D'Souza and Schellenberg 2002) (Fig. 2C). This splicing modulator is disrupted by the exon 10 +19 mutation and results in increased 3R tau isoforms (Stanford et al. 2003). These two elements seem to act in opposition and it has been suggested that trans-acting factors that bind the modulator sterically hinder other trans-acting factors from attaching to the silencer, which in turn allows access to the 5' splice site (Fig. 2C).

Tauopathy mutations

In contrast to FTDP-17, PSP is overwhelmingly a sporadic disease. There have been, however, a few individuals identified carrying coding *MAPT* mutations who present clinically with symptoms similar to those seen in sporadic PSP. It is thought in these rare cases that the *MAPT* mutations do account for the isoform imbalance seen in aggregates, or otherwise affect the aetiology of the disease. In one patient with PSP-like clinical symptoms and neuropathology, a dominant silent mutation AGT > AGC (S305S) has been identified (Stanford et al. 2000) (Fig. 2A). S305S falls within the putative stem loop structure and its presence results in over a four-fold increase in exon 10 inclusion and subsequent over-production of 4R tau (Stanford et al. 2000). A recessive mutation, a deletion at N296 (N296del) generates an atypical PSP phenotype (Pastor et al. 2001) and falls in a region defined as a splicing silencer (D'Souza and Schellenberg 2000). An exonic splicing enhancer is altered by the G303V mutation, carriers of which show greater exon 10 + transcripts than controls (Ros et al. 2005).

An interesting mutation generating a PSP-like phenotype is the R5L mutation located in *MAPT* exon 1 (Poorkaj et al. 2002). Aggregated insoluble tau in the R5L case was comprised of predominantly 4R tau isoforms similar to sporadic PSP, however, the amount of soluble tau in the frontal and temporal cortices case was 1.5 – 2 times higher than both sporadic PSP and controls. In addition, the mutation altered the interaction of the mutant protein with tubulin and microtubules. It has been suggested that the N-terminal substitution exerts its effect on microtubule binding through interaction of the N-terminal and the microtubule binding domains in paired helical filaments but not normal tau (Carmel et al. 1996; Poorkaj et al. 2002).

The diverse nature of *MAPT* mutations indicates the complex interplay of regulatory sequences in intron 9, exon 10 and intron 10 to maintain tau isoform ratios. Much current research is now focused on the natural sequence variation in the region of *MAPT* and how this variation may confer susceptibility over time to neurodegenerative disease.

MAPT haplotype association with sporadic tauopathies

The MAPT H1 and H2 Haplotypes

The genetic link between *MAPT* and PSP started with the identification of an association between PSP and a polymorphic marker found in *MAPT* intron 9 (Conrad et al. 1997) (Fig. 3B). Both the dinucleotide repeat allele A0 and the genotype A0/A0 are over-represented in PSP; for example, one study found A0/A0 is carried in 57% of controls compared to 95% in PSP patients (Conrad et al. 1997). Further investigation of the polymorphisms over-represented in PSP led to the elucidation of an extended haplotype covering the entire *MAPT* locus (Baker et al. 1999) (Fig. 3B). Eight common single nucleotide polymorphisms (SNPs) across *MAPT* were identified and found to be in complete linkage disequilibrium (LD) with each other and the A0 allele. The more common *MAPTH1* haplotype was found to be associated with PSP. A 238 bp deletion was also discovered to be inherited as part of the less common H2 extended haplotype (Fig. 3B). The extended H1 haplotype was found to have a frequency of 94% in PSP patients compared to only 78% in controls (Baker et al. 1999). The *MAPTH1* haplotype association with PSP has been consistently replicated in different Caucasian populations (Conrad et al. 1997; Oliva et al. 1998; Baker et al. 1999; Morris et al. 1999). Interestingly, the H2 haplotype is absent in Japanese populations (Conrad et al. 1998; Evans et al. 2004) and is thought to be exclusively Caucasian in origin (Evans et al. 2004). In addition to being associated with PSP, the H1 haplotype is also shown to be over-represented in CBD, another of the 4R tauopathies (Di Maria et al. 2000; Houlden et al. 2001).

The H1 haplotype defined by Baker et al (1999) at 100 kb was initially expanded to include the promoter (de Silva et al. 2001; Pastor et al. 2002) and now has been refined to span a region covering ~1.8 Mb, in which all SNPs in LD show association to PSP (Pittman et al. 2004; Pittman et al. 2005). This region is relatively gene rich, with the *CRHR1* (corticotrophin) and *IMP5* (a presenilin homologue) genes at the centromeric end of LD, while *WNT3* and *NSF* (N-ethylmaleimide-sensitive factor) at the telomeric end of the LD. (Pittman et al. 2004) (Fig. 3A).

Recently, it has been found that a ~900 kb segment from H2 chromosomes is inverted with respect to the H1 haplotype (Stefansson et al. 2005) (Fig. 3A). This inversion is thought to have occurred between 3 and 3.6 million years ago (Stefansson et al. 2005) and is almost entirely absent outside of European populations (Evans et al. 2004). The relative homogeneity of the H2 haplotype and the higher frequency in Europeans is consistent with a few founder chromosomes, probably expanding due to positive selection (Stefansson et al. 2005).

Further investigations into the genomic architecture of this region have identified a number of low-copy repeat (LCR) sequences (Cruts et al. 2005). There is evidence to suggest that the non-allelic homologous recombination that generated the inversion at H2 occurred between two low-copy repeat sequences, LCR A and LCR B, located 250 kb centromeric and 180 kb telomeric to *MAPT* respectively (Cruts et al. 2005). Non-allelic homologous recombination between LCRs has also been suggested to be the cause of a recently identified microdeletion encompassing *MAPT* in patients with learning disability and developmental delay (Koolen et al. 2006; Shaw-Smith et al. 2006; Varela et al. 2006).

In addition to the previously defined polymorphisms, a complex arrangement of duplicated regions was also discovered along with the inversion to segregate with *MAPT* haplotypes (Stefansson et al. 2005). Four H1 surrogate or sub-haplotypes have been defined with respect to the size, location and dosage of these regions, H1_{D0} – H1_{D3}. The H1_{D3} variant has a triplication of the first 13 exons of *NSF*, while the H1_{D0} variant has a duplicated region not

containing NSF. Recently, a study of the global variation of copy number in the human genome has identified population-specific copy number polymorphisms in the genomic region surrounding *MAPT* (Redon et al. 2006). The number of segmental duplications, segmental duplication inversions and copy number polymorphisms near *MAPT* highlights the complexity of this genomic region.

MAPT H1 Sub-haplotypes

Exhaustive sequence analysis in the *MAPT* genomic region has also further divided the H1 haplotype into subtypes and subsequently allowed the fine mapping of the *MAPT* association with PSP (Pittman et al. 2005; Rademakers et al. 2005). In addition to H1 and H2 haplotype defining SNPs, there exist SNPs that vary only on the H1 background, H1-specific SNPs (Pittman et al. 2004). Using 5 haplotype tagging polymorphisms (rs1467967, rs242557, rs3785883, rs2471738, rs7521) and the 238 bp haplotype defining insertion-deletion to capture 95% of the common haplotype diversity, the population has been divided into several sub-haplotypes for association analysis (Fig. 3B). In both UK and US cohorts, the H1C haplotype, a sub-clade of H1, is over-represented in PSP patients compared to controls (Pittman et al. 2005). Moreover, strong association of haplotype sub-class II defined by three H1-specific haplotype tagging SNPs (rs242557, rs3785883, rs2471738), suggests variability within the H1 clade itself is a risk factor for PSP (Pittman et al. 2005). The fine mapping of these SNPs indicates the association is conveyed by a region covering a minimal distance of ~54 kb from upstream of exon 1 to downstream of exon 9 (Pittman et al. 2005).

Interestingly, another study also shows a strong association to rs242557 with an 11.6% increase of the same 'A' allele in PSP patient populations over controls (Rademakers et al. 2005). The highest significance in this study was obtained in LD block 2, part of an interval spanning ~22 kb of regulatory sequence upstream of exon 1 (Rademakers et al. 2005). Both sub-haplotype studies indicate greatest association of PSP with the regulatory region of *MAPT*, making the promoter region a good candidate for functional studies.

Haplotype promoter strength

Promoter strength assayed by reporter gene

Several studies have found strong association between susceptibility to neurological disorders and specific alleles or haplotypes of candidate genes (Bray et al. 2003; Bray et al. 2004; Bray et al. 2005; Paracchini et al. 2006; Urak et al. 2006). In the absence of protein coding changes, these investigations have focused on relevant non-coding polymorphisms and how these polymorphisms regulate gene expression. For example, one study investigating the genetic basis of dyslexia found association to one risk haplotype that results in ~40% reduction in expression of *KIAA0319* (Paracchini et al. 2006). Subtle changes in the relative gene expression between risk and non-risk alleles have been suggested as a functional mechanism whereby a risk haplotype confers susceptibility to disease.

Recent PSP association studies propose that variants in the *MAPT* promoter region are responsible for susceptibility to PSP. One theory to explain the association of H1 with PSP proposes that there are overall expression differences between the two haplotypes. To investigate this one study employed reporter genes downstream of 1 kb promoter fragments of the H1, H2 or H1' haplotypes to assay promoter strength (Kwok et al. 2004). H1' is a promoter haplotype differing from H1 by one SNP 568 bp upstream of exon -1. In HEK 293 kidney and SKNMC neuroblastoma cell lines the H2 promoter showed a 1.2-fold reduction in transcriptional activity compared to the H1 promoter. In SKNMC cells, the H1' promoter also showed a 1.1-fold decrease in transcriptional activity compared to the H1 promoter (Kwok et al. 2004). While these differences in promoter strength may be small, it is

proposed that over time, the small increase in expression from the H1 haplotype may lead to disease.

Another assay analysed a conserved region containing the haplotype tagging SNP rs242557 (Rademakers et al. 2005), which has been shown to be associated with PSP (Pittman et al. 2004; Pittman et al. 2005; Rademakers et al. 2005). *In silico* the risk variant was predicted to abolish binding sites for trans-acting factors, Se-Cys tRNA gene transcription-activation factor (STAF.01) and CP2-erythrocyte Factor related to drosophila Elf1 (CP2). The proposed regulatory region was placed upstream of both a minimal SV40 early promoter and a 1.1 kb fragment of the *MAPTH1* promoter. Under these conditions, the non-risk allele showed greater transcriptional activity (Rademakers et al. 2005). In contrast, another study using this same SNP placed downstream of the *MAPT* promoter region showed that the H1 haplotype construct expresses 4.2 fold greater expression than the non-risk H2 promoter (Myers et al. 2007).

The differing results from these promoter assays are likely because their experimental designs use small fragments of regulatory sequence isolated from their correct genomic context. The study that found the non-risk regulatory region had greater transcriptional activity placed a small 182 bp fragment containing the SNP from exon 0, upstream of a promoter (Rademakers et al. 2005). Using a more biologically-relevant design, the function of the same SNP was assayed using a 900 bp fragment in the correct relative linear order to the *MAPT* promoter, although with the removal of ~50 kb of intervening sequence (Myers et al. 2007).

Whole locus analysis of MAPT expression

Several studies have tried using systems that assay expression from the whole *MAPT* genomic locus. Real-time PCR analysis of tau expression in post-mortem human brain tissue showed individuals carrying the homozygous H1C haplotype have a three to four fold greater expression compared to promoters of all other genotypes (Myers et al. 2007). This data agrees with data obtained from reporter gene assays but carries greater in vivo validity due to the whole locus analysis that is possible in human tissue.

Powerful tools to investigate the effect of sequence variation on transcript expression are found in technologies that allow allele-specific or haplotype-specific gene expression studies (Yan et al. 2002; Knight 2004). The strength of these techniques derives from their ability to analyze allele-specific gene expression within a heterozygous sample eliminating the effects of confounding factors between samples such as sample quality, environmental effects and genetic background.

Two studies have used allelic-specific gene expression techniques to address the question of *MAPT* promoter haplotype strength. A haplotype tagging SNP in intron 1 was used to assay haplotype specific expression in heterozygous samples (Caffrey et al. 2006). Significantly greater expression from the H1 promoter was seen in both SKNF1 and SKNMC neuroblastoma cell lines, similar to the findings in reporter gene assays (Kwok et al. 2004; Caffrey et al. 2006; Myers et al. 2007) However, across 14 heterozygous, pathology-free, post-mortem human brain samples, there was no allelic difference observed in expression in either the frontal cortex or globus pallidus. In another study examining the effect of sub-haplotype on expression, allele-specific real-time PCR showed that the H1C haplotype has a significant 11-13% greater expression compared to all other *MAPT* haplotypes examined (Myers et al. 2007).

Specific tau isoform expression

Another potential mechanism by which tau may confer susceptibility to neurodegeneration is through the imbalanced expression of alternative transcripts, leading to imbalanced protein isoform species. The importance of tau isoform balance is highlighted by FTDP-17 mutations which increase the inclusion of exon 10 in transcripts, resulting in a two to six fold excess of 4R tau mRNA (Hutton et al. 1998; Spillantini et al. 1998; D'Souza et al. 1999; Connell et al. 2005). The isoform imbalance in FTDP-17 is sufficient to cause disease, and suggests the possibility that isoform imbalance may be a contributing factor to sporadic tauopathies.

The first indication of altered tau mRNA isoform expression in PSP was demonstrated using real-time PCR to examine expression in PSP patient tissue (Chambers et al. 1999). The cerebellar cortex, a region free of NFTs in PSP, was analyzed and showed a significant decrease in 3R mRNA levels, thus increasing the 4R/3R ratios in this region. In the frontal cortex, a region with mild NFT pathology in PSP, there was no significant difference in mRNA levels for either 4R or 3R tau (Chambers et al. 1999). Finally, in the highly affected brainstem region, there was a significant increase in 4R tau mRNA in PSP patients compared with controls and AD cases. This suggests that the ratio of splice products is important for correct function.

In support of the a role of isoform imbalance in areas susceptible to degeneration and in the aetiology of sporadic 4R tauopathies, Takanashi et al (2002) found the total amounts of 3R and 4R tau transcript did not differ significantly between controls and patients, however the ratio of 4R:3R was significantly higher in the globus pallidus of PSP patients and the 4R:3R ratio is also higher in both the frontal cortex and globus pallidus in CBD patient tissues (Takanashi et al. 2002). Moreover, a study of pathology free controls showed a significantly higher 4R:3R mRNA transcript ratio in the globus pallidus than in a frontal cortex, a region not affected by neurodegeneration in PSP (Caffrey et al. 2006). This elevated ratio of 4R:3R evidence suggests that the expression pattern of tau isoforms in brain regions vulnerable to neurodegeneration in PSP may underlie the susceptibility of these areas to develop neurodegeneration.

In addition to certain brain regions being more susceptible to disease, functional evidence is emerging to suggest why H1 haplotype carriers may also be susceptible. Using allele-specific expression assays in differentiated H1/H2 heterozygous neuroblastoma cell line models, significantly greater expression of exon 10+ transcripts was shown coming from the H1 chromosome. When the assay was performed in pathology-free post-mortem human brain tissue, H1 chromosomes were shown to express significantly more exon 10+ transcripts than H2 chromosomes, in both the frontal cortex and globus pallidus (Caffrey et al. 2006). This difference in expression was most pronounced in the globus pallidus where H1 expresses up to 40% more 4R mRNA transcripts. In a similar study where exon 10+ transcripts were analyzed according to sub-haplotype, a significant 25% greater expression of 4R transcripts was seen originating from H1C chromosomes (Myers et al. 2007). These are key findings that start bridging the gap between the H1 haplotype association of 4R tauopathies such as PSP and CBD and the neuropathology seen in affected areas.

Conclusion

Recent investigations elucidating the complex genomic architecture of chromosome 17q21 and fine mapping of the linkage disequilibrium spanning the *MAPT* gene locus now place us in a good position to start assessing the functional effects of *MAPT* haplotype. Data from various experimental approaches are converging to reveal subtle differences in haplotype

expression, which over time may lead to neurodegenerative disease. The relatively greater expression of *MAPT* exon 10+ transcripts from the PSP-associated H1 haplotype is of particular relevance because 4R tau isoforms predominately form the abnormal tau aggregates in 4R tauopathies. For the first time, we are now able to link the neuropathology observed in PSP patients with the well-established genetic association to the *MAPT* H1 haplotype and to begin shedding light on how natural variation may confer susceptibility to neurodegeneration.

However, dissecting the precise functional genetic mechanisms and identifying the exact polymorphisms responsible for regulation of *MAPT* expression and splicing remains a huge challenge. Within the *MAPT* locus alone there are over 400 documented SNPs (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) indicating the difficulties in locating the functional polymorphisms. In common with other complex genetic diseases we should expect the functional influences of common genetic polymorphisms at the *MAPT* locus to be subtle and cumulative over many years. The challenge for future studies is to design functional experiments to study the biological relevance of genetic polymorphisms within the context of the genomic DNA locus.

Acknowledgments

This work was supported by a Research Career Development Fellowship awarded to RW-M from the Wellcome Trust and by a Clarendon Fund Bursary awarded to TMC.

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Figure 1.

MAPT locus consists of 16 exons of which exons 2, 3 and 10 are alternatively spliced in the adult CNS (grey or black stripes). *Top*: Exons 2 and 3 code for the N-terminal projection domain (black stripes). Exons 9 –12 imperfect repeats each coding for a microtubule-binding domain (grey). Exons 4A and 8 (white) are absent from the CNS, but exon 4A is expressed in the peripheral nervous system. *Bottom*: Six tau isoforms are expressed in the adult CNS. Alternative splicing of exons 2 and 3 results in proteins with 0, 1 or 2 N-terminal inserts (ON, 1N, 2N). Splicing of exon 10 generates proteins with either 3 or 4 microtubule-binding repeats (3R or 4R tau protein).

Figure 2.

Cis-elements affecting splicing of *MAPT* exon 10. Silencer (red) and enhancer (green) sequences within and surrounding exon 10 are indicated. Exon 10 sequence is designated by capital letters and intronic sequence is indicated by small case letters. (A) Several cis-elements affect *MAPT* exon 10 splicing, listed 5' to 3': a SC35-like enhancer, a polypurine enhancer (PPE), an A/C-rich enhancer (ACE), an exonic splicing silencer (ESS), an exonic splicing enhancer (ESE), an intronic splicing silencer (ISS) and an intronic splicing modulator (ISM). Select FTDP-17 and PSP mutations are also shown. (B) Alternative splicing regulation at the tau exon 10 5' splice site. Two of the variant stem-loop structures proposed to regulate splicing at the tau exon 5' splice site (Hutton et al. 1998; Spillantini et al. 1998). (C) The linear model of tau exon 10 5' splicing regulation postulates that binding of trans-acting factors to the cis-regulatory elements mediates exon 10 alternative splicing. Shown here is a trans-acting factor bound to the ISM. This ISM binding protein sterically hinders a trans-acting silencer (ISS binding protein) from binding the silencing cis-element, thereby allowing access of the U1 snRNP to the 5' splice site (D'Souza and Schellenberg 2002; D'Souza and Schellenberg 2005).

Figure 3.

MAPT Haplotypes. (A) The *MAPT* locus has been divided into two major haplotypes, H1 and H2. *MAPT* falls within the largest known block of LD in the human genome, spanning ~1.8 Mb. There is a 900 kb inversion of the H2 haplotype with respect to the H1 haplotype, covering a region containing *MAPT*, *IMP5*, *CRHR1* and *NSF*. (B) Association of the dinucleotide marker A0 in *MAPT* intron 9 to PSP was the first genetic link between the disease and tau. This association was extended to cover the entire locus, using a 238 bp deletion (+/- 238 bp) and 8 SNPs (SNP 1 – SNP 13) to tag the haplotypes (Baker et al. 1999). Sub-haplotypes of H1 have since been identified using several haplotype tagging SNPs (rs1467967, rs24557, rs3875883, rs2471738, rs7521) (Pittman et al. 2004).